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Pharmacological Approaches to Inhibit Endogenous Glucose Production as a Means of Anti-diabetic Therapy

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Abstract: The inappropriate overproduction of glucose by the liver is one of the key contributors to the hyperglycaemia of the diabetic state, and thus is a logical site of intervention for novel anti-diabetic approaches. Metformin is the only currently marketed anti-hyperglycaemic drug whose action is attributed largely to its having inhibitory effects on hepatic glucose production, but its molecular site and mechanism(s) of action remain unknown, whereas the liver acting PPARα agonists have their effects primarily on lipid metabolism. This review therefore rather focuses on candidate molecular targets within the liver for anti-hyperglycaemic therapy, and describes potential rate-controlling receptors and enzymes within the glucose producing pathways (glycogenolysis and gluconeogenesis). Most focus is directed towards inhibitors of the enzymes glucose-6-phosphatase, fructose-1,6-bisphosphatase and glycogen phosphorylase, and towards glucagon receptor antagonists, as these appear to be the most advanced in preclinical and clinical development, although progress with other potential targets is also described. Evidence of the anti-diabetic potential of such agents from animal studies is presented, and the relative merits of each approach are reviewed and compared. It is likely that such agents will become important additions to the therapeutic approaches to combat diabetes.

INTRODUCTION

The concentration of glucose in the blood is dictated by the relative rates of glucose utilisation and of glucose production by the body tissues [1-3]. The brain is responsible for most of the glucose uptake in the resting state, and the rate of this is relatively constant under different states of feeding and is not significantly affected by insulin [4], whereas the muscle is the most significant insulin-responsive site of glucose utilisation after a meal [1-3], although the liver can still account for around 30% of the glucose disposal following carbohydrate ingestion [1-3]. The kidney will only produce significant amounts of glucose after severe starvation, and therefore the liver is normally the only tissue producing glucose, and this is under a process that is sensitive to insulin [1-3]. Therefore it can be argued that liver glucose production is perhaps the most important site at which insulin regulates blood glucose concentration [1-3].

In normal healthy individuals the blood glucose concentration is closely controlled at around 5mM. Type 2 diabetes is a heterogeneous disorder characterised by hyperglycaemia, which is the result of both impaired insulin secretion and of insulin resistance in target tissues [5]. The

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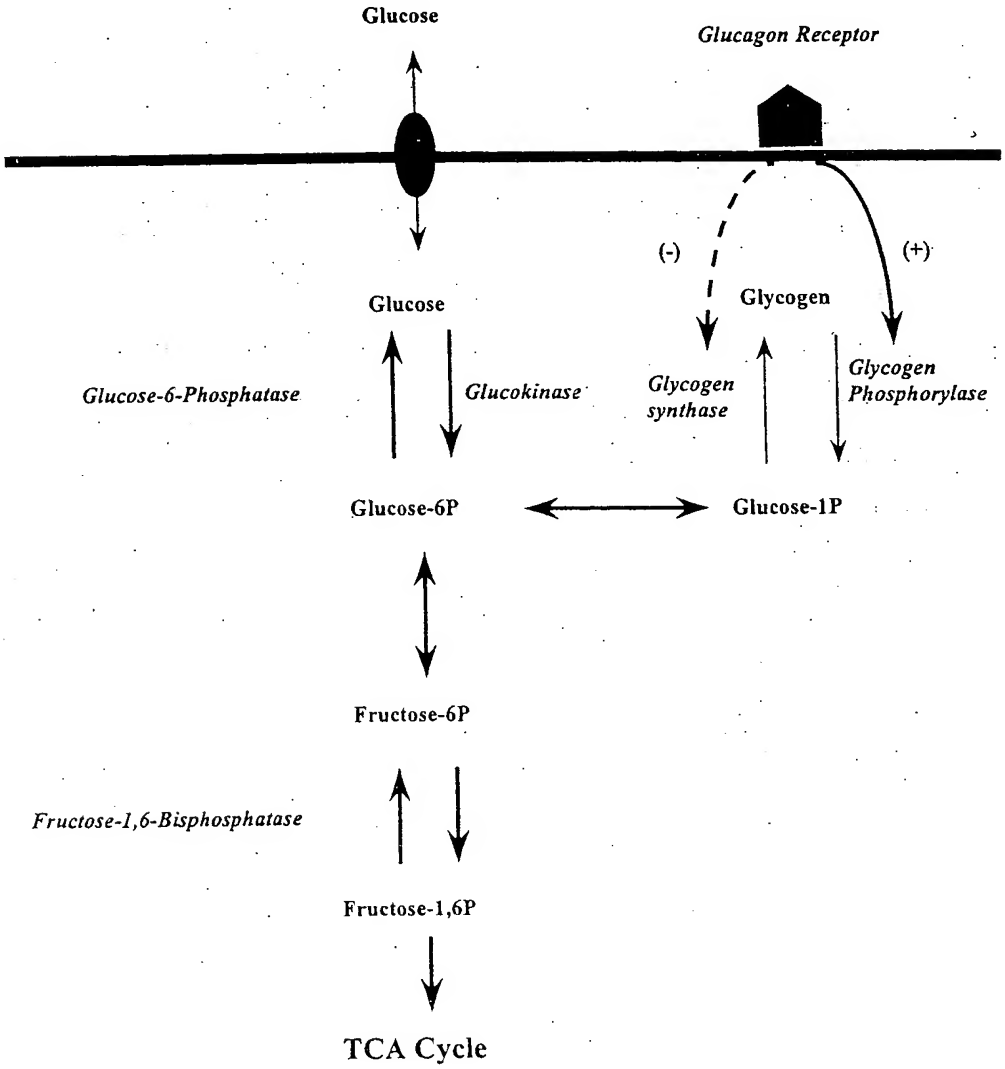
resistance to insulin action is mainly manifest at the muscle (decreased glucose clearance) and liver (reduced suppression of glucose production), both of which contribute to the hyperglycaemic state [5], although the primary site of action of the insulin sensitizer class of drugs (the "thiazolidinediones" such as troglitazone, rosiglitazone and pioglitazone) is agonism of the PPARγ intracellular receptor in adipose tissue [6]. Numerous studies have shown that hepatic glucose production (HGP) is increased in type 2 diabetes in the post-absorptive state, and that this is directly correlated with fasting hyperglycaemia (e.g. [7-10]). Moreover, it is now increasingly recognised that also in the post-prandial state the impaired suppression of HGP is a main contributor to, and perhaps the major mechanism responsible for, the increased hyperglycemia of the diabetic state [1-3, 5].

There is little doubt therefore, that increased HGP plays a primary role in establishing the hyperglycaemia of the type 2 diabetic patient in both the post-absorptive and post-prandial states, and thus the development of new drugs to decrease HGP may offer new forms of treatment for this disease. The two major pathways for HGP are glycogenolysis (the breakdown of the glucose polymer glycogen) and gluconeogenesis (the synthesis of glucose from 3-carbon precursors) (Fig. 1). It has proven difficult to obtain accurate and definitive measurements of each of these pathways in humans, and as a result there is considerable debate in the literature as to which



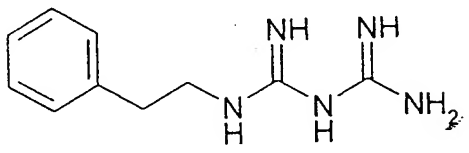
may play a predominant role, or for that matter be the most attractive to target for drug development, in the diabetic state (eg [1-3, 11-13]). In fact it may be that the development of specific pharmacological tools directed towards one pathway or the other may be the best way to answer such questions. Therefore, there is currently drug discovery and development activity directed towards both pathways individually and collectively, as is shown in Fig. **1**, and as is discussed in more detail later below for each particular target. But first, there should be mention of metformin, which is a marketed drug for which inhibition of HGP is thought to be its main mechanism of action [14, 15].

METFORMIN

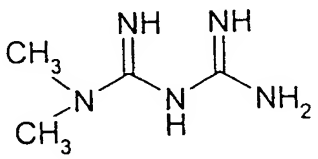
This drug is a biguanide (Fig. **2**), which has been used in the treatment of type 2 diabetes for over four decades [14, 15], and therefore there is a vast amount of clinical experience with the drug. The United Kingdom Prospective Diabetes Study (UKPDS, see [16]) and many other studies (see [14, 15]) have clearly shown that it is an effective anti-diabetic therapy, with, however, some side effects and contra-indications [14, 15]. However, its cellular mechanism of action and its molecular target(s) are not known, and many different actions on different tissues have been described, although its main effect is often cited as being in the reduction of both hepatic gluconeogenesis and glycogenolysis [13-15]. This lack of a molecular target has meant that there has been little optimisation of this drug which needs to be taken in gram quantities, and the only other biguanide developed for anti-diabetic therapy, phenformin (Fig. 2), had to be withdrawn because of safety concerns regarding lacticacidosis [14, 15]. Thus further development from metformin itself has been restricted to formulation development, and a long-acting form (Glucophage®XR) and a formulation in conjunction with glyburide, a sulphonylurea insulin-secretagogue (Glucovance™), have recently been introduced by Bristol-Myers-Squibb.

An interesting new hypothesis as regards to metformin's primary site of action, which is unifying in that it could explain many of the described effects of the drug on different parameters in different tissues, has recently been advanced [17]. This is that metformin acts as a weak, and self-limiting, inhibitor of the mitochondrial respiratory Complex I. The proposal is that there is a slow mitochondrial-membrane potential driven accumulation of the drug (due to its modest lipophilicity and positive charge) within the mitochondrial matrix leading to inhibition of Complex I, particularly in the liver, where this would lead to reductions in ATP production and thus energy-requiring gluconeogenesis, and in peripheral tissues where it would lead to glucose uptake to drive anaerobic ATP production; increased lactate production can thus also be explained in this way [17]. In this regard, several other weak respiratory chain inhibitors were shown to produce metabolic effects in liver preparations that were similar to those of metformin [17]. The self-limitation in metformin's action would arise from the fact that progressive inhibition of the respiratory chain would lead to a drop in mitochondrial membrane potential, which would then prevent further accumulation of the drug. In contrast, the phenylethyl group on phenformin (Fig. 2) makes it a more lipophilic drug that can permeate membranes more readily, and thus this may explain its greater toxicity potential [17].

The only other drugs currently used in treatment of (some) type 2 diabetic patients that have their primary site of action at the liver are the PPARα agonists [18] such as fenfibrate, however, as these have their primary effect on lipid metabolism and not on HGP *per se* they will not be discussed further in this review.



Phenformin



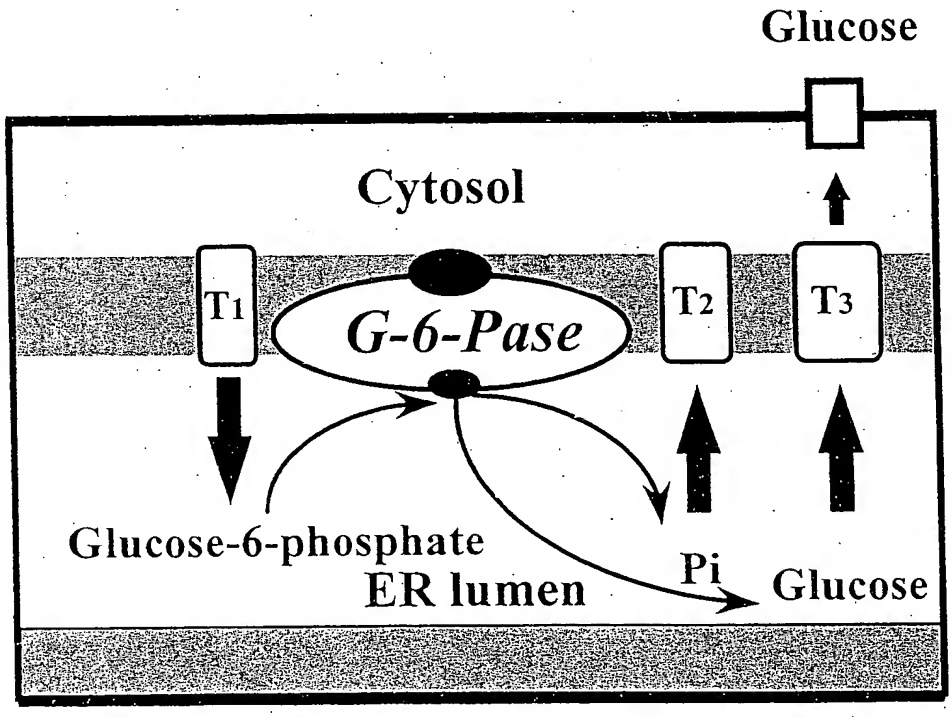
Metformin

POTENTIAL TARGETS FOR INHIBITION OF HGP

As discussed above (Fig. 1), inhibition of key enzymes in the glycogenolytic and gluconeogenic pathways is an obvious approach, as is inhibition of the action of the counter-regulatory hormone to insulin, i.e. glucagon. Thus inhibition of glucose-6-phosphatase, fructose-1,6-bisphosphatase and glycogen phosphorylase have been approached as means of inhibiting HGP, and progress with these will be described in turn below, as will the antagonism of the action of glucagon. (The development of inhibitors against the first two targets just mentioned would also be expected to inhibit the contribution of the kidney to overall glucose production.) A converse approach would be to upregulate the activity of glucokinase (GK) in the liver (Fig. 1) [19], which could perhaps be achieved by inhibition of its interaction with its inhibitory regulatory protein, but there have been no publications in using this approach. Likewise, the upregulation of glucokinase expression, or the downregulation of gluconeogenic enzymes expression has been proposed as an approach, but there is as yet little literature reporting any advances in this area.

GLUCOSE-6-PHOSPHATASE INHIBITION

Glucose-6-phosphatase (G-6-Pase), which is predominantly located in the liver [20], catalyses the terminal step in both gluconeogenesis and glycogenolysis by converting glucose-6-phosphate (G-6-P) to glucose and inorganic phosphate [21, 22] and is, therefore, a key regulating step in blood glucose homeostasis [23] (Fig. 1). This has been demonstrated in patients with glycogen storage disease type 1 [24, 25], where the enzyme is deficient causing, amongst many clinical features, hypoglycemia. In contrast, in diabetic animal models the catalytic enzyme activity [26-29], protein content [28] and mRNA levels [26, 27] are all increased and presumably contribute to the prevailing hyperglycaemia. It has also been shown that over-expression of the G-6-Pase catalytic protein in cultured hepatocytes resulted in metabolic profiles resembling those seen in the livers of diabetic animals and patients [30], i.e. less glycogen and G-6-P content, and increased glucose production. Thus, since G-6-Pase seems to play a pivotal role in the increased hepatic glucose production seen in type 2 diabetes, this enzyme constitutes a potential target for the treatment of the disease.



However, in order to design new chemical entities targeting this enzyme the structure function-relationship of this multi-component enzyme should be considered. Currently, two hypotheses exist for this enzyme: the "substrate transport model" and the "combined conformational flexibility-substrate transport model" (see [23]). The former model is the most widely accepted and the one that has been used to design G-6-Pase inhibitors. According to the substrate transport model (Fig. 3) G-6-Pase is comprised of: 1) the G-6-Pase catalytic protein with its active site located at the luminal side of the endoplasmic reticulum (ER), 2) a specific transporter T_1 (T_1-translocase) which mediates the entry of G-6-P into the luminal compartment, and 3) transporters T_2 and T_3 which mediate the export back to the cytosol of inorganic phosphate and glucose respectively.

Since entry of G-6-P into the lumen of the ER via T_1-translocase is the rate-limiting step in phosphohydrolysis [31], this translocase is an obvious target for pharmacological intervention. Another approach in targeting this enzyme could be to inhibit the G-6-Pase catalytic protein, since it is this protein [26-29], rather than the T_1-translocase, that is increased several-fold in diabetes, and therefore in all likelihood is responsible for the increased G-6-Pase activity and consequential over-production of glucose in the disease. Recently, Hoechst Marion and Roussel (now Aventis) and Novo Nordisk have published *in vitro* and *in vivo* data on inhibitors of the T_1-translocase and the G-6-Pase catalytic protein, respectively, which will now be dealt with in the following sections.

T_1-translocase Inhibitors

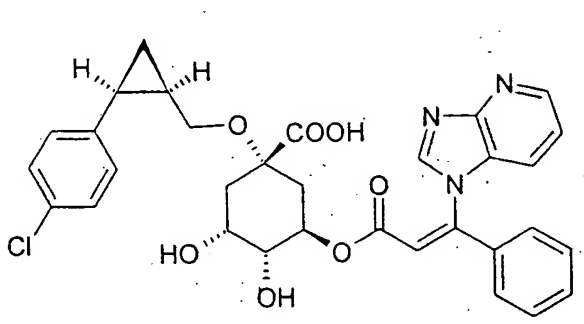
Several inhibitors of the T_1-translocase have been reported [32, 33], however, it was not until recently that chlorogenic acid was reported as the first specific T_1-translocase inhibitor with an IC_{50} value of 226 μM on rat liver microsomes [33, 34]. S-3483, a chlorogenic acid derivative, has subsequently been shown to be a specific and potent inhibitor of the T_1-translocase with an IC_{50} value of 210 nM in rat liver microsomes [35]. In non-diabetic animal models [36], this compound in a high dose could reduce blood glucose levels with a concomitant increase in blood lactate levels and in intra-hepatic concentrations of glycogen and G-6-P, consistent with an inhibitory effect on G-6-Pase. The pharmacological effect of another chlorogenic acid derivative, S-4048 (Fig. 4), has also been shown in ob/ob mice and normal rats [37, 38]. In ob/ob mice and their control littermates dosed with S-4048, a markedly improved glucose tolerance was achieved, and this effect was not related to plasma insulin levels, as insulin levels actually decreased [37].

G-6-Pase Catalytic Protein Inhibitors

The findings that vanadate [39], tungstate [40] and peroxyvanadium compounds [41] are potent inhibitors of the G-6-Pase catalytic protein suggest that the blood glucose lowering effects of these compounds, which have been observed in diabetic animals, may at least partly be explained by a direct effect on this enzyme rather than, at least for vanadate and peroxyvanadium compounds, as is presently thought, of this being a result of inhibition of phosphoprotein tyrosine phosphatases [41]. This suggests that inhibition of the G-6-Pase catalytic protein could also be of potential interest as an anti-diabetic drug target.

We have reported previously a series of 4,5,6,7-tetrahydrothieno pyridines as highly potent G-6-Pase catalytic protein inhibitors with functional IC_{50} values down to 140 nM using disrupted pig liver microsomes [42]. Further characterisation [43] of the active enantiomer of 4-methoxyphenyl-[4-(4-methoxyphenyl)-4, 5, 6, 7-tetrahydrothieno [3,2-c]pyridin-5-yl]methanone (NNC-60-0452) (Fig. 4) from this series of compounds showed that this compound, in a competitive mode of action, was able to inhibit G-6-Pase activity in both intact and disrupted pig microsomes more potently than in rat microsomes. In addition, this compound was able to inhibit basal and glucagon stimulated glucose production from cultured rat hepatocytes in the low μM range with a concomitant 2-fold increase in G-6-P content, consistent with the expected mechanism of action.

Altogether, it is concluded that inhibitors of the T_1-translocase as well as of the G-6-Pase catalytic protein could be of therapeutic importance in the search for anti-hyperglycaemic agents for the treatment of type 2 diabetes, providing that



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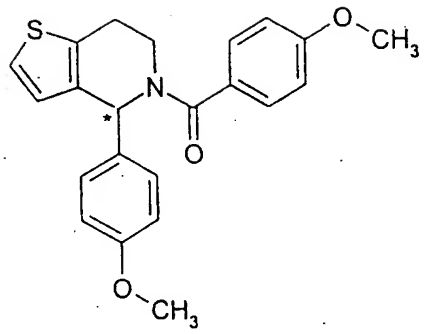


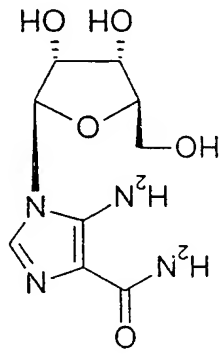
NNC 60-0452

Fig. (4). Structures of the T_1-translocase inhibitor S-4048 and of the G-6-Pase catalytic inhibitor NNC 60-0452. *Indicates an asymmetric carbon.

compounds with proper pharmacokinetic and pharmacodynamic properities can be identified.

FRUCTOSE-1,6-BISPHOSPHATASE INHIBITORS

This enzyme (F-1,6-BPase) catalyses one of the key rate-limiting steps in the gluconeogenic pathway (Fig. 1), and numerous studies have shown that the overall gluconeogenic process is upregulated in the type 2 diabetic, and is thus likely to contribute significantly to the hyperglycaemic state (e.g. [1-3, 5, 11-13]). A useful tool in probing the effects of inhibition of this enzyme has been AICAriboside (AICAR: 5-aminoimidazole-4-carboxamide riboside (Fig. 5)) which is the nucleoside corresponding to AICA-ribotide (or ZMP: 5-aminoimidazole-4-carboxamide ribonucleotide monophosphate), an intermediate of the *de novo* pathway of purine nucleotide biosynthesis. The latter has been shown to be an inhibitor of F-1,6-BPase, as the result of its structural similarity to the physiological allosteric non-competitive inhibitor AMP, and the



former (AICAR) (Fig. 5) is able to cross cell membranes and, upon intracellular phosphorylation, thus deliver the inhibitor to this cytosolic enzyme (see [44]). However, use of this agent has been complicated by the recent demonstration that ZMP also activates the AMP-dependent protein kinase, and AICAR is now being widely used to probe the role that this enzyme plays in processes that also have implications for the diabetic state. For example, it is now proposed that activation of this enzyme is one of the key steps in the contraction-mediated stimulation of glucose uptake in muscle [45, 46].

Nevertheless, it can be assumed that at least some of the acute effects of AICAR administration reside in its leading to the production of ZMP in liver cells and inhibition of F-1,6-BPase. Thus it has been shown to result in potent, dose-dependent inhibition of gluconeogenesis in isolated hepatocytes [47], and blood glucose lowering in animals [44], including in rodent models of diabetes where efficacy could be shown without increases in blood lactate levels [48, 49]. Moreover, in agreement with its mechanism of action in this regard, increases in liver cell or tissue concentrations of fructose-1,6-bisphosphate could be demonstrated [47-49]. Metabasis Inc. (ex-Gensia Inc.) has been at the forefront of developing novel inhibitors of this enzyme using structure-based approaches employing X-ray crystallography and site-directed mutagenesis [50, 51], and based on novel structures targeting the AMP pocket. This has involved design of novel analogues of AICAR that will access the intracellular compartment and become phosphorylated there, but this company has also

prodrugs as potential intracellular delivery systems for phosphonates [52].

Thus F-1,6-BPase inhibitors have also been shown to have *in vivo* efficacy in animal models of diabetes and thus also represent a potential approach to combat this disease.

GLYCOGEN PHOSPHORYLASE INHIBITORS

As already mentioned (Fig. **1**), the inappropriate production of glucose from hepatic glycogen is also implicated in contributing to the hyperglycaemia of the diabetic state, and thus glycogen phosphorylase as the rate-limiting enzyme in this process has been addressed as a target.

Glycogen Metabolism

Glycogen serves in most mammalian cells, but particularly in liver and muscle, as a metabolic fuel reservoir for glucose [53]. In tissues such as the brain and red blood cells a constant supply of glucose is essential, since these tissues depend entirely on glucose as the sole energy source for glycolysis. Mobilisation of glycogen from the liver provides a constant supply of glucose when required, and thus plays an important role in maintaining a plasma glucose level of 5-6 mM, whereas mobilisation in muscle cells provides glucose only for their own intracellular use.

Glycogen breakdown or glycogenolysis requires three enzymes [53]: 1) glycogen phosphorylase which catalyses the phosphohydrolysis to yield glucose-1-phosphate; 2) glycogen debranching enzyme which removes glycogen branches thereby making them accessible to GP; and 3) phosphoglucomutase to convert glucose-1-phosphate to glucose-6-phosphate.

Glycogen phosphorylase (GP), which is believed to exist as a dimer, is, as already stated the rate-limiting step in the overall process of glycogenolysis and displays normal Michaelis-Menten kinetics [54]. There are three isozymes of mammalian GP [53, 55], all encoded by different genes located on (human) chromosomes 20, 11, 14, and designated brain, skeletal muscle and liver isozymes respectively, according to the tissues in which they are preferentially expressed.

In the liver, control of the rate of glycogen synthesis and glycogen mobilisation is accomplished by covalent modification of glycogen synthase and GP by phosphorylation and de-phosphorylation processes. Hepatic glycogen mobilisation is stimulated by glucagon and adrenaline via a cascade of events (Fig. 6), resulting in the phosphorylation (at serine 14 in each subunit) and promotion of the activation of GP [56]. De-phosphorylation of GP is mediated by protein phosphatases, including Protein Phosphatase 1 (PP1) [57, 58]. The phosphorylated form of GP is termed GPa and the non-phosphorylated form is termed GPb. Both

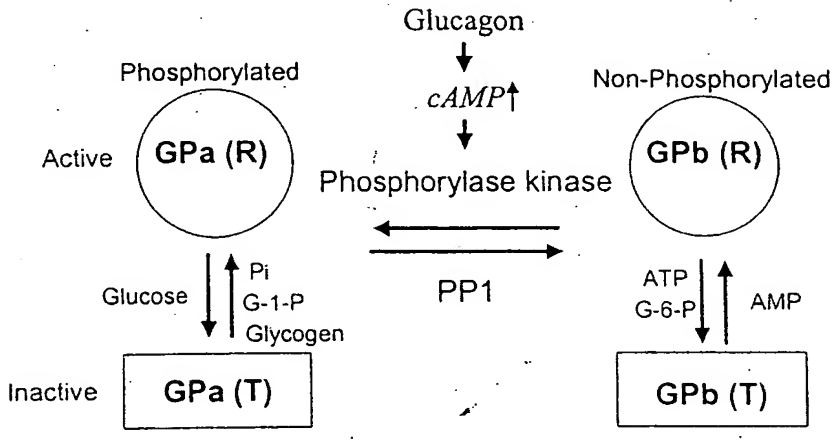


Fig. (6). Schematic diagram showing the regulation of GP activity. Stimulation with glucagon increases the level of cAMP, which leads, by a series of phosphorylation events, to activation of phosphorylase kinase. This enzyme converts the non-phosphorylated form GPb into its phosphorylated form GPa. The two conformational states, T-state and R-state, are shown as

forms can exist in an inactive conformation, the T-state, and an active conformation, the R-state [59].

Besides covalent modification, GP can also be regulated by allosteric effectors (see also next section). As with F-1,6-BPase, a major regulator is AMP which can activate the muscle GPb isozyme to 80% of the GPa activity [55] by stabilising the more active R-state [60], whereas AMP only activates the GPb liver isozyme to 20% of the GPa activity [61]. GPa does not require AMP for activation; however, AMP is able to increase the GPa activity by 10-20% [59].

GP inhibitors – Different Classes of Compounds

In order to inhibit GP and so to reduce glycogenolysis, it is reasonable to propose that an inhibitor that binds to GP and stabilises the enzyme in the T-state conformation could be of major importance in finding new drugs for the treatment of type 2 diabetes. In the literature there are several articles describing compounds binding to GP with such features, which will be dealt with in the following sections.

There are five known binding sites in the GP enzyme: the catalytic site, the inhibitor (or nucleoside) site, the AMP allosteric (or nucleotide) site, the glycogen storage site, and a new allosteric inhibitor site that was identified recently as a target for drug interactions (Fig. 7); these are now dealt with in turn.

Catalytic Site Inhibitors

The catalytic site is situated approx. 15 Å from the surface of the enzyme. Glucose as well as glucose-1-phosphate, and a number of other glucose analogues (Fig. 8), inhibit GP by binding to this site and stabilising the enzyme in the T-state conformation [62-69], and thereby also making GPa more susceptible to de-phosphorylation and de-activation by PP1. Pyridoxal phosphate is an essential co-factor for binding of these ligands to GP. Spirohydantoin (1) and spirothiohydantoin (2) (Fig. 8) are the most potent compounds

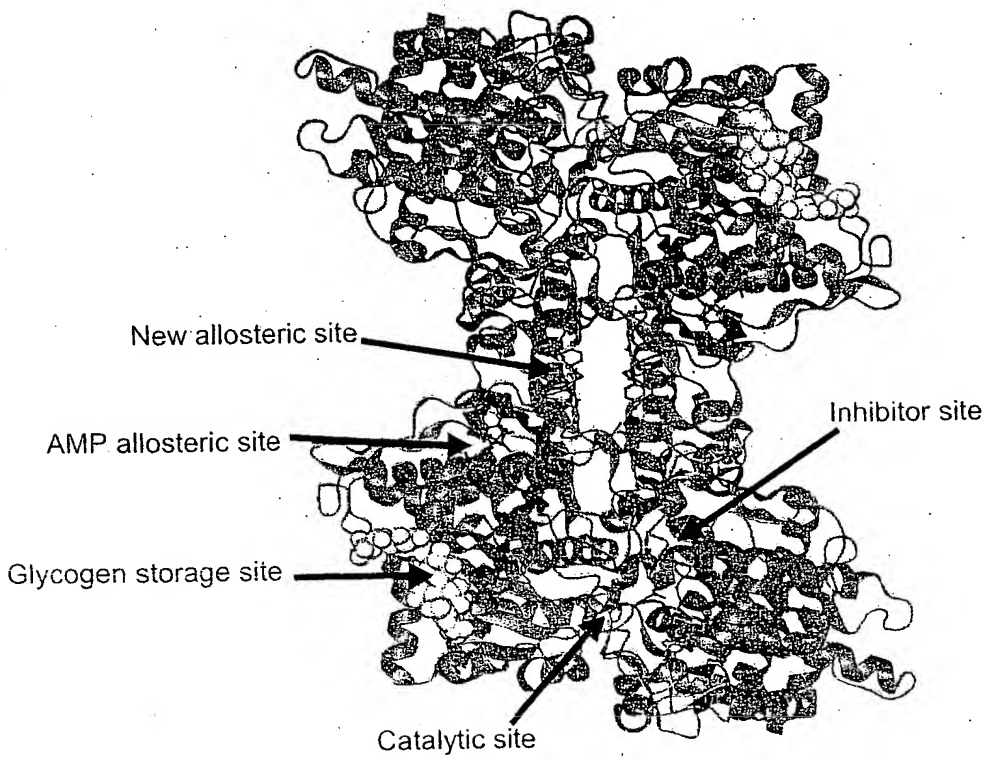


Fig. (7). A schematic representation of the dimer of rabbit muscle GPb showing the five binding sites: the catalytic (active) site, the inhibitor (or nucleoside) site, the AMP allosteric (or nucleotide) site, the glycogen storage site, and the new allosteric

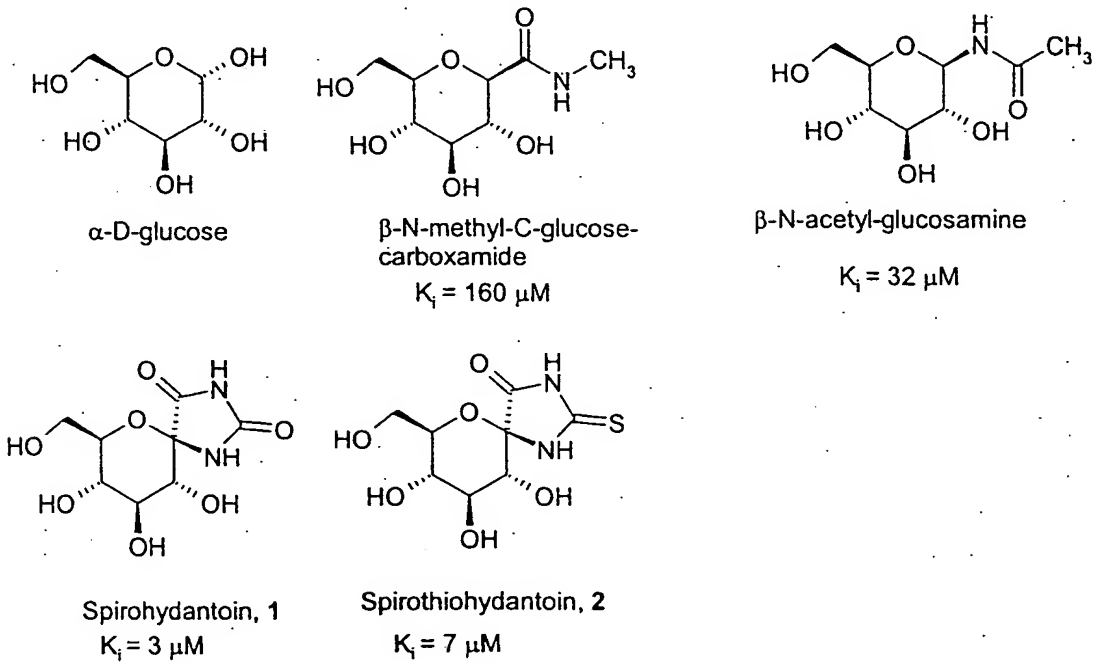


Fig. (8). Examples of compounds binding at the catalytic site of GP. K_i values are obtained using GPb from rabbit muscle measured in direction of glycogen synthesis. The corresponding K_i value for glucose itself is 1.7mM.

reported within this series, with K_i values of 3 and 7 μM, respectively.

The glucose analogue 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) (Fig. 9) has recently been shown to be a potent inhibitor of both forms of GP obtained from various sources, when GP was measured in the direction of glycogenolysis [70]. In spite of its resemblance to glucose and glucose analogues also inhibiting GP [63, 64, 71], this compound had several striking differences when the mode of action was compared to those other compounds [70]. Thus, the inhibitory mode of action of DAB was found to be un- or non-competitive [70] as opposed to that for glucose and the other glucose analogues, which were found to be competitive inhibitors [66]. In addition, AMP was able to abolish the inhibition of the glucose analogues [66], whereas AMP had no effect on the potency of DAB [70]. It was also found that DAB, unlike glucose and the other analogues [67], reversed the inhibitory effects of caffeine [70]. Moreover, glucose did not affect the potency of DAB when GP was measured in the direction of glycogenolysis. This suggests that DAB, from a mechanistic point of view, defines a novel mechanism of action compared to other known GP inhibitors. In this context, it should also be mentioned that isofagomine, which is a structural analogue of DAB (Fig. 9), was also found to be a potent inhibitor of GP with similar inhibitory characteristics to DAB [72, 73].

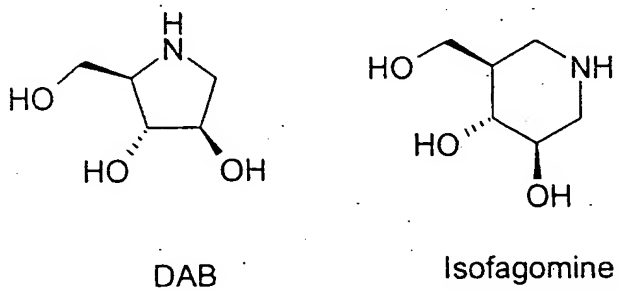


Fig. (9). DAB and isofagomine, which have been shown to be potent GP-inhibitors with IC_{50} values of 0.2 and 0.8 μM respectively on pig liver GPa.

Using cultured rat hepatocytes, DAB has been shown to inhibit basal and glucagon-stimulated glucose production with IC_{50} values of 1-2 μM [74], and this was shown also to be related to the concomitant prevention of glycogen degradation, confirming that glycogenolysis was inhibited. Furthermore, by using ^{13}C-NMR, DAB has been shown to prevent glucagon-induced glycogen degradation in livers of ob/ob and lean mice [70]. Thus, glycogen levels were 361 and 228 μmol glucose units/g in lean and ob/ob mice treated with

DAB and glucagon, compared to 115 and 37 μmol glucose units/g when treated with glucagon alone (after 2.5h). Moreover, end-point blood glucose levels were found to be 29 and 18 mM in obese and lean mice treated with glucagon compared to 18 and 12 mM in mice treated with glucagon and DAB, thus confirming the data obtained in cultured hepatocytes.

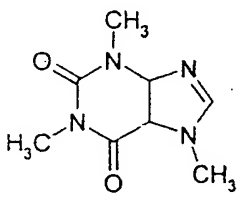
It has been proposed that glycogenolysis and gluconeogenesis are interrelated in such a way that changes in flux in one pathway may be compensated for by a compensatory increase in flux in the other pathway [75, 76], resulting in an unchanged net endogenous glucose production (EGP). By infusion of DAB into overnight fasted dogs it could be shown that DAB inhibited glucagon-stimulated glycogenolysis by 46% resulting in an inhibition of EGP by 21 %, and, significantly, without affecting the flux from gluconeogenesis, suggesting that inter-regulation of glucose production may not take place when glycogenolysis is inhibited [77]. This example also shows how having a precise pharmacological tool can allow important physiological and patho-physiological questions to be approached and resolved.

The Inhibitor Site

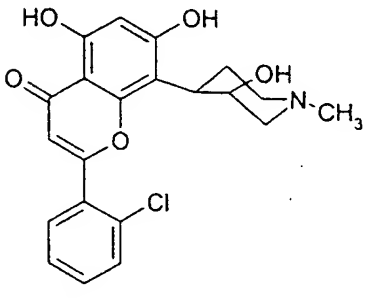
The inhibitor (or nucleoside) site is located at the entrance to the catalytic site, near the surface and approximately 10 Å from the catalytic site (Fig. 7). This site binds different aromatic compounds, such as caffeine and flavopiridol, ((-)-cis-5,7-dihydroxy-2-(2-chlorophenyl)-8-[4S-(3R-hydroxy-1-methyl)piperidinyl]-4H-benzopyran-4-one), which is a potential anti-tumor drug [78] (Fig. **10**). Flavopiridol is reported to inhibit rabbit muscle GPb with an IC_{50} value of 15.5 μM when measured in the direction of glycogen synthesis, and 1 μM when measured in the direction of glycogen breakdown. Kinetic data revealed that flavopiridol inhibition of GPb is synergistic with glucose, suggesting that both flavopiridol and glucose are able to bind to the GP enzyme at the same time. Flavopiridol inhibits the enzyme by stabilizing the T-state conformation, as do caffeine and glucose. Caffeine is reported to inhibit both liver and muscle GPa in synergism with glucose as well, and with a K_i value of approx. 1 mM [67, 79-81].

The AMP Allosteric Site

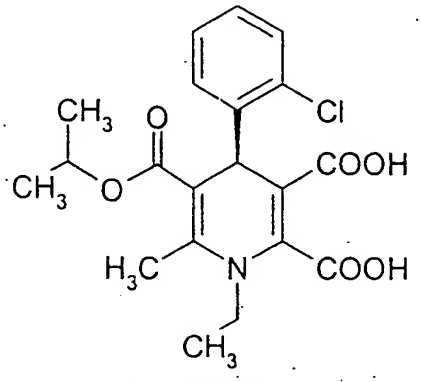
The AMP allosteric (or nucleotide) site, which is located more than 30 Å from the catalytic site (Fig. 7), binds a variety of phosphorylated compounds, such as AMP, ATP, IMP, and glucose-6-phosphate, as well as a dicarboxylic pyridine derivative, (-)(S)-3-iso-propyl-4-(2-chlorophenyl)-1,4-dihydro-1-ethyl-2-methylpyridine-3,5,6-tricarboxylate, from Bayer (Bay W1807) (Fig. **11**) [59, 82-84]. Bay W1807 was found to induce conformational changes upon binding to the protein, which locks the protein in the inactive T-conformation. The dihydropyridine derivative Bay R3401 is metabolised to Bay W1807, which has been found to be a potent inhibitor of muscle GPa and GPb [59, 83] with K_i values of 10.8 and 1.6 nM, respectively. Kinetic studies revealed that Bay W1807 was competitive with respect to glucose-1-phosphate [59] and AMP [83], and acted in synergism with glucose, similar to that shown for caffeine [79]. In hepatocytes, and in perfused livers, Bay W1807 suppressed glycogenolysis by allosteric inhibition and by the de-phosphorylation of GPa [85]. In the



Caffeine

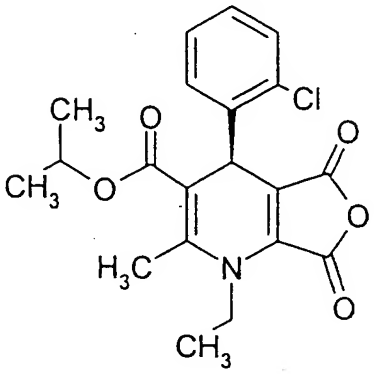


Flavopiridol



Bay W1807

K_i = 1.6 nM



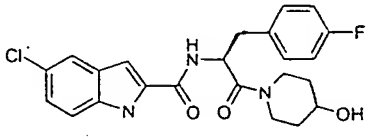
Prodrug of Bay W1807
(Bay R3401)

Fig. (11). Examples of compounds binding to the AMP allosteric site of GP. The K_i value was obtained from GPb rabbit muscle measured in direction of glycogen synthesis. The prodrug has been found to be orally active in dogs [82].

livers of conscious dogs, Bay R3401 has been shown to cause a marked reduction in basal and glucagon-stimulated glycogenolysis, and as a consequence a reduction of EGP [82]. As a result of these changes, gluconeogenesis was augmented by increased uptake of gluconeogenic precursors and increased glycogen synthesis was also observed [82].

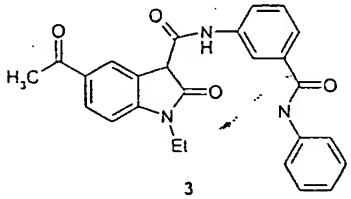
New Allosteric Binding Site

A new allosteric site has recently been reported [86] to be located at the subunit interface in the region of the central cavity of the dimeric structure (Fig. 7). This site is situated approx. 15 Å from the AMP allosteric site, 37 Å from the inhibitor site, and 33 Å from the catalytic site. Two different indole derivatives have been demonstrated to bind at this site, namely 5-chloro-1H-indole-2-carboxylic acid (1(S)-(4-fluorobenzyl)-2-(4-hydroxypiperidin-1-yl)-2-oxo-ethyl)amide, (CP-320626), and different 2-oxo-2,3-dihydro-1H-indole-3-carboxylic acid amides, e.g. (3), from Pfizer (Fig. 12) [87]. Oikonomakos *et. al.* [86] reported that CP-320626 inhibits the GPb muscle enzyme by stabilizing the T-state conformation. Recently, another indole analogue, CP-91149 (Fig. 12), has been shown to be a potent inhibitor of human liver GP [88]. Moreover, the potency of CP-91149 was found to be glucose-dependent in a similar way as for caffeine [89], in that these compounds were found to be five-fold less potent in the absence of glucose. This effect of glucose on the potency of CP-91149 could have some clinical implications in the treatment of diabetes, since the potency of these types of compounds decreases as the glucose concentrations go down, and thereby treatment with such agents would theoretically diminish the risk of hypoglycemia, a major concern in type 2 diabetes therapy. However, the physiological relevance of this is questionable since the changes in potency are only gained in the range of 0-6 mM glucose. Most notably, the studies using CP-91149 also provided strong supporting evidence for the concept that a GP inhibitor would also lead to inhibition of gluconeogenesis, and for the concomitant hypothesis that a substantial part of the flux through the gluconeogenic pathway also cycles through glycogen (see [89]), thus increasing the apparent attractiveness of GP as a drug target.



CP-320626

K_i = 334 nM



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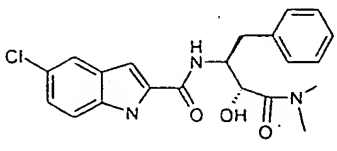


CP-91149

Fig. (12). Examples of compounds binding at the new allosteric binding site in the region of the central cavity of the GP

Chronic treatment of fed ob/ob mice with CP-320626 for 15 days resulted in a 20-35% reduction in plasma glucose levels throughout the treatment period [90]. This effect was reflected in a 30% increase in liver glycogen content that had reached a plateau after 5 days of treatment, substantiating the expected inhibition of GP. In this study no data was given on HbA1c, however, the elevated levels of triglycerides, cholesterol, insulin and lactate found in ob/ob mice were reduced by 26-48% by this compound [90].

The Glycogen Storage Site

The last binding site described so far, the glycogen storage site, is located on the surface of the enzyme (Fig. 7) and binds, apart from glycogen and malto-oligosaccharide, also acarbose, an α-glucosidase inhibitor from Bayer [91, 92]. Acarbose, a pseudo-tetrasaccharide, is an even poorer GP inhibitor than glucose with a K_i value of 26 mM on rabbit muscle GPa. The crystal structure data show that it binds to the T-state conformation of the enzyme in the presence of both glucose and caffeine [78].

Thus overall there are a number of potential different ways of inhibiting GP, and progress has been achieved based on the structural detail known about the different inhibitory sites, and already there is a considerable body of data suggesting *in vivo* efficacy of GP inhibitors in animal models of diabetes. Pfizer has reported that they are in Phase II development with a GP inhibitor (CP 368,296), and thus it should soon be evident if such an approach will be useful in treating diabetic patients.

GLUCAGON RECEPTOR ANTAGONISTS

Glucagon was among the first hormones to be isolated and characterized during the late 1950's and the 1960's [93]. At that time, Unger *et al.* developed a radio-immunoassay for detection of circulating glucagon [94, 95] that was made available for the scientific community, initiating a more than 30 years period of research into the physiology of this hormone [93]. The liver is the primary target organ for glucagon, and its function in the regulation of hepatic glucose metabolism is mediated through binding to its specific receptor

Under normal conditions, when the body is in need for glucose as fuel (e.g. during fasting and exercise), glucagon concomitantly stimulates glycogen breakdown and *de novo* synthesis of glucose (both via activation of cyclic AMP-dependent and possibly also non-cyclic AMP-dependent pathways), and also inhibits glucose storage into glycogen (by inactivation of glycogen synthase), three actions which together lead to mobilization of glucose by the liver (detailed reviews in [96-98]). The proposed patho-physiological role for glucagon in the development and maintenance of hyperglycaemia in diabetic patients, however, has often been a matter of controversy, which viewed retrospectively has, at least to some extent, been due to a lack of appropriate tools to bring about experimental conditions of *selective* glucagon deficiency or excess. According to the "bihormonal abnormality" hypothesis, proposed by Unger and Orci some 25 years ago [99], diabetes is a bihormonal disorder in which relative or absolute lack of insulin, together with an excess of glucagon, cause impairment of peripheral glucose metabolism and overproduction of glucose by the liver.

Somatostatin has been widely used as a tool to suppress endogenous glucagon secretion in humans and experimental animals as a way to study its normal and pathological physiology under various conditions. Insulin, however, is also suppressed by somatostatin, and the resultant effects on blood glucose under such conditions, therefore, depends on the extent to which insulin has been replaced. Because endogenous glucagon may naturally influence insulin secretion, it is impossible to predict what the insulin levels, and hence the blood glucose responses, would have been during selective glucagon deficiency. In addition, somatostatin also has other actions involved in glucose homeostasis, such as on the absorption of nutrients from the gastrointestinal tract [100], regulation of splanchnic blood flow [101], and enhancement of hepatic glucose production [102], all of which make it even more difficult to interpret the results from such studies. However, although it has been technically difficult to demonstrate glucagon's patho-physiological role directly, several reports leave not much doubt that glucagon plays an important role in the pathogenesis of diabetes. For example, it has been shown that hyperglucagonaemia co-exists with hyperglycaemia throughout the day in both lean and obese type 2 diabetics [103], and that elevated glucagon

is involved in the maintenance of increased hepatic glucose output in type 2 diabetes [104]. More recently, K. Larson and B. Ahrén reported from a prospective study [105] in which they have followed post-menopausal women over 3 years. This study showed that, of the many parameters measured, increased glucagon secretion was the strongest predictor of development of glucose intolerance, suggesting that elevated glucagon is a major patho-physiological factor in the etiology of type 2 diabetes.

On this background it seems appropriate to inhibit the actions of glucagon in diabetic patients. However, so far no glucagon antagonists have been available for clinical trials in diabetic patients. Therefore, in an attempt to obtain a clearer picture of what effects on blood glucose regulation could possibly be expected *in vivo* of an ideal, selective, glucagon antagonist, we developed a high-affinity monoclonal anti-glucagon antibody that has proven to be extremely efficient at immuno-neutralising both exogenously administered, as well as endogenous, glucagon in experimental animals [106]. The corresponding monoclonal control-antibody used in control studies was considered, and shown to be, "inert". We have thus used these antibodies to demonstrate the potential of selective glucagon "antagonism" in the treatment of experimental diabetes, however, being also well aware that the mechanism of action of these antibodies is different from that of a glucagon antagonist that acts at the level of the glucagon receptor, and that animal models of diabetes only

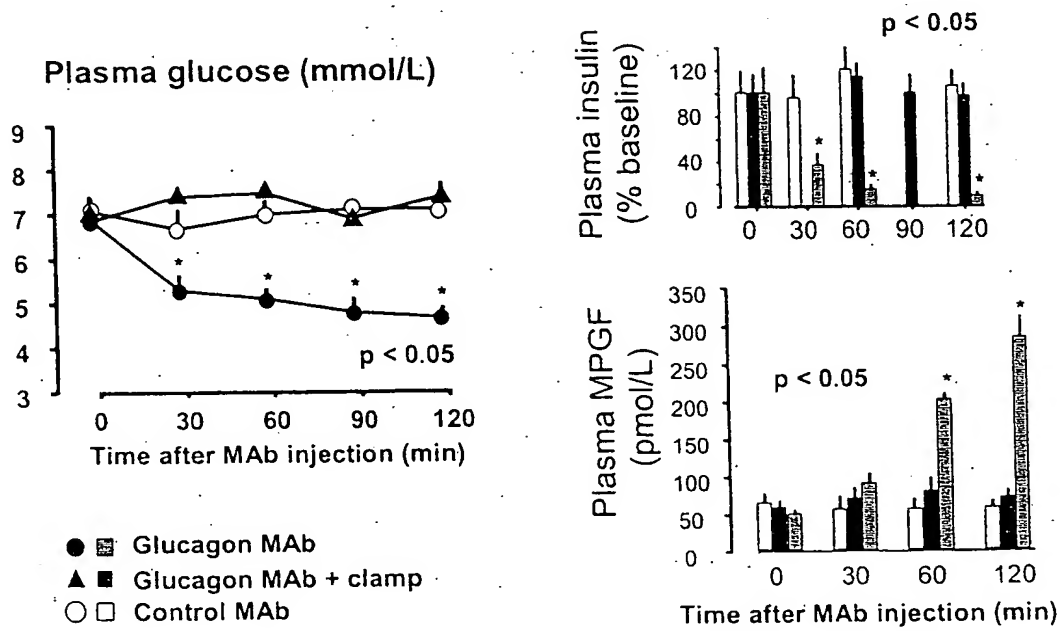


Fig. (13). The figure shows plasma glucose (left panel), insulin (right, top panel) and major pro-glucagon fragment (MPGF; surrogate measure of glucagon secretion) levels (right, bottom panel) in post-absorptive rabbits injected intravenously with monoclonal control antibody (○ and open bars) and monoclonal glucagon antibody with (▲ and hatched bars) and without (● and solid bars) being subjected to euglycaemic clamp. It is demonstrated that immunoneutralization of endogenous glucagon causes a marked drop in plasma glucose whereas insulin and glucagon secretion decreases and increases, respectively, solely as a result of the change in glucose and not as a result of the glucagon "deficiency". Data are mean±SE, n=6-8 per group; *indicates significant differences from the corresponding control MAb data. Reproduced with permission from *Diabetes*, ref. [107].

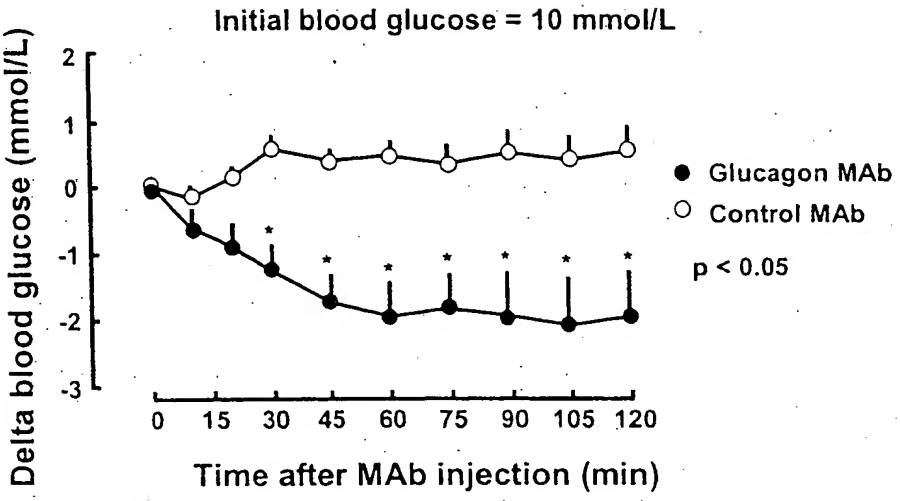


Fig. (14). The figure shows blood glucose levels in streptozotocin-induced severely diabetic rats (negligible endogenous insulin secretion preserved) that have been pre-treated with insulin for one week after streptozotocin in order to partially restore euglycaemia (~10 mmol/l) prior to an intravenous injection with monoclonal control antibody (○) or with monoclonal glucagon antibody (●). It is demonstrated that blood glucose is reduced by 2 mmol/l in the diabetic rats when glucagons is removed. Data are mean±SE, n=9 per group; *indicates significant difference between matched values. Reproduced with permission from *Diabetologia*, ref. [108].

resemble human diabetes in a limited number of aspects.

In general, and for the first time ever using this approach to obtain selective glucagon deficiency, we demonstrated that glucagon plays an important role in the maintenance of glucose homeostasis in normal rats and rabbits (see Fig. 13), and moreover, that hypoglycaemia does not develop despite combined blockade of circulating glucagon and catecholamines [106, 107]. More importantly, glucagon immunoneutralisation was shown to

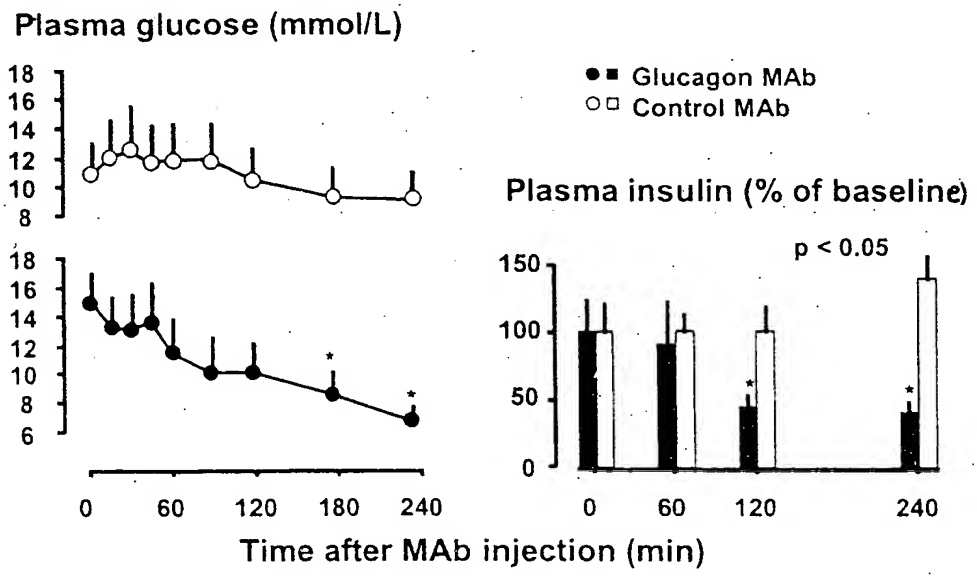


Fig. (15). The figure shows plasma glucose (left panel) and insulin levels (right panel) in all9xan-induced moderately diabetic rabbits (sub-optimal endogenous insulin secretion preserved) injected intravenously with monoclonal control antibody (○ and open bars) and monoclonal glucagon antibody (● and solid bars). Is is demonstrated that blood glucose is reduced by up to 8 mmol/l in diabetic rabbits. Data are mean±SE,, n=5 per group; *indicates significant difference between matched values. Reproduced with permission from *Diabetologia*, ref. [107].

reduce hyperglycaemia acutely (2-6 hours) in streptozotocin-diabetic rats [108] (see Fig. **14**), and in alloxan-diabetic rabbits [107] (see Fig. 15). Furthermore, in the ob/ob mouse, which is characterized by genetical leptin deficiency causing obesity, hyperphagia, hyperinsulinaemia, as well as moderate hyperglycaemia and hypertriglyceridaemia, both acute (6 hours) and long-term (5-14 days) glucagon immunoneutralisation reduced hyperglycaemia [109]. Over the longer term, glucagon immunoneutralisation in ob/ob mice also reduced HbA1c (compared to control-treated ob/ob mice) and hypertriglyceridemia [109].

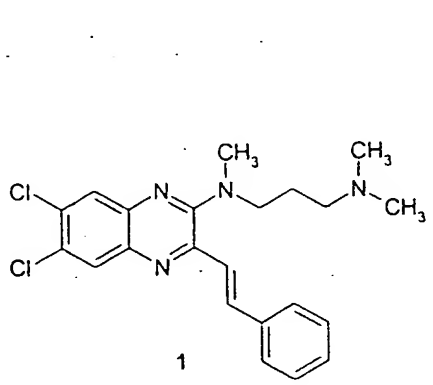
Taken together, these results strongly suggest that glucagon antagonism may represent a beneficial approach in the treatment of diabetes. However, it should be borne in mind that HGP is regulated in a complex way by several hormones other than glucagon, as well as by nerves and substrate availability. Moreover, elevated HGP is not thought to be solely responsible for the diabetic syndrome [1-3, 5]. It has been speculated, therefore, that even if complete glucagon antagonism reduces HGP by maximally 25-50%, and that if this reduction *per se* does not revert the remaining abnormalities also present in the diabetic state, a complete normalization of hyperglycaemia should perhaps not be expected as a result of monotherapy treatment with a glucagon antagonist [106]. Conversely, however, the data obtained with glucagon immunoneutralisation in animals [106-109] (Figs. **13-15**) indicate that it may indeed provide efficacious first line monotherapy.

Therefore, on account of the evidence detailed above, several pharmaceutical companies have shown public interest in the field by publishing their work on the development of glucagon antagonists to be used for the treatment of diabetes, as will now be described.

Non-peptide Glucagon Antagonists

Several reviews [110-112] have been published on the topic of glucagon receptor antagonists. Although peptide glucagon antagonists are known, we will in the present context only summarise on glucagon antagonists of non-peptidyl origin.

The first publication [113] on non-peptide ligands for the glucagon receptor appeared in 1992 from a group at Pfizer Inc. describing CP-99,771, a styryl quinoxaline (**1**) (Fig. **16**) with glucagon receptor antagonistic properties. The compound was reported to displace ^{125}I-glucagon from isolated rat liver membranes at low micromolar concentrations (IC_{50} = 4μM), and to inhibit glucagon-stimulated cAMP formation (IC_{50} = 7μM). The authors speculated that the compound binds to a common domain in G-protein coupled receptors (GPCRs) that is most likely unspecific [113]. Another group [114] has since reported on constrained derivatives of CP-99,711, preparing a series of pyrrolo[1,2-a]quinoxalines. The most potent compound (**2**) (Fig. **16**) had a binding affinity (IC_{50}) of 5μM in a RIN T3, β-cell line assay. For comparison, CP-99,711 had an IC_{50} value of 0.1μM in this assay. These data



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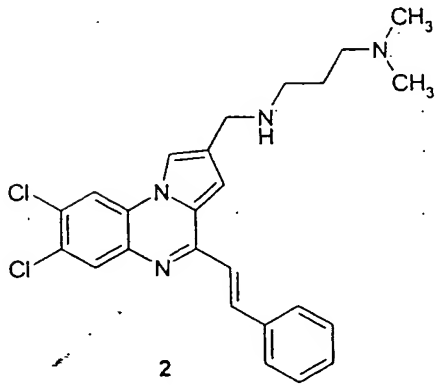
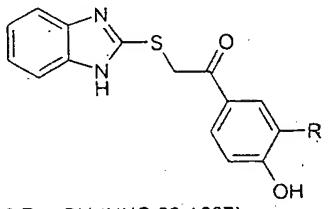


2

Fig. (16). Binding affinity of (1) [CP-99,711] in isolated rat glucagon receptor assay: IC_{50} = 7μM; on RIN T3, β-cell line assay: IC_{50} = 0.1μM. Binding affinity of (2) in RIN T3, β-cell line assay: IC_{50} = 5μM.

demonstrate that in general it is not possible to compare IC_{50} values of glucagon receptor antagonists across different binding assays.

A research group at Novo Nordisk [115] has reported catechol [3,4-dihydroxyphenyl] derivatives to be competitive glucagon antagonists. A structure-activity relationship (SAR) on this series of compounds was investigated. Based on derivatives of the original hit (3) (Fig. 17), NNC 92-1687 [2-(benzimidazol-2-sulfanyl-1-(3,4-dihydroxyphenyl)-1-ethanone], it was found that most structural changes resulted in loss of binding affinity. However, the 3-hydroxy group of the catechol moiety could be exchanged to a methoxy or chloro group with retained affinity to the hGluc receptor [115]. The most active analogues within this series are mercaptobenzimidazoles (Fig. 17), which have affinity in the micromolar range towards the human glucagon receptor measured in baby hamster kidney (BHK) cells transfected with hGlucR. The compounds were found to be competitive and specific antagonists, inhibiting glucagon but not forskolin stimulated cAMP formation, again using recombinant human glucagon receptors in BHK cells.



3 R = OH (NNC 92-1687)
4 R = Cl
5 R = OMe
6 R= H

Fig. (17). Binding affinities of mercaptobenzimidazoles (3) (NNC 92-1687), (4), (5), and (6) in the baby hamster kidney (BHK) cells transfected with hGlucR, IC_{50} values respectively, 20μM, 12μM, 41μM and >60μM.

De Laszlo *et al.* [116] have published the discovery of orally available 2-pyridyl-3,5-diaryl pyrroles with glucagon antagonistic properties. The initial hit (7) (Fig. 18) was an imidazole analogue found by screening of the Merck collection of compounds in a competitive human glucagon binding assay using ^{125}I-glucagon as radioligand in Chinese hamster ovary (CHO) cells transiently expressing the hGluc receptor. The initial hit (7) was a p38 kinase inhibitor (p38 IC_{50} = 117nM) with weak hGluc receptor affinity (IC_{50} = 490nM). During the optimisation of the imidazole series it was found that the isomeric pyrroles had improved affinity towards the hGluc receptor compared to the imidazole series. The affinity of both these series of glucagon antagonists were reduced five- to ten-fold in the presence of physiological Mg^{++} concentrations. By modifying the properties and pattern of the pyrrole substituents, the group at Merck optimised the affinity and selectivity of these pyrroles towards the hGluc receptor [116].

Pyrroles lacking a 5-substituent, or having small alkyl groups in this position, had diminished hGluc receptor affinity, but maintained the p38 affinity. However, by introduction of larger alkyl or aryl substituents at the 5-position, they obtained compounds with improved affinity towards the hGluc receptor. 4-Chlorophenyl was found to be an optimal 5-substituent, and e.g. in compound (9) (Fig. 18) high hGluc receptor affinity (IC_{50} = 80nM (-Mg^{++}), 800nM (+Mg^{++})), and improved selectivity compared to (7) (p38 IC_{50} = 1400nM), was obtained. It is worth noting that significant species variation with respect to binding towards the glucagon receptor was observed for compound (9), in that it did not bind the glucagon receptors isolated from rat, guinea pig and rabbit livers, whereas affinity towards the dog and mouse glucagon receptors was found (IC_{50} = 80nM and 200nM (-/+ Mg^{++}), respectively).

The 3-substituent was optimised in a new sub-series where the shape and electronic properties were found to be important for both affinity and selectivity towards the hGluc receptor. 2-Propoxy-5-bromophenyl (Fig. 18) was an optimal 3-substituent giving rise to a selective glucagon receptor ligand (10) (hGlucR IC_{50} = 7nM (-Mg^{++}), 170nM (+Mg^{++}); p38 IC_{50} = 1440nM), which was a non-competitive hGluc antagonist inhibiting glucagon (100pM) stimulated cAMP production in the transfected CHO cells. In a Schild-plot analysis, the slope was 0.6 and K_b = 25nM. Even though the compound was orally available in mice and rats, there have not been published any pharmacodynamic data related to this compound.

In a separate publication [117], a comprehensive biochemical characterisation of compound (10) (Fig. 18), L-168,049 has been

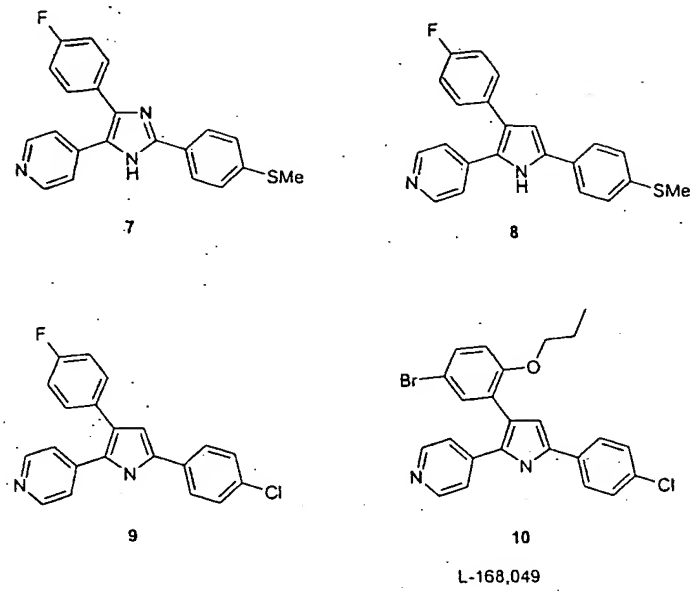


Fig. (18). Binding affinity of (7), (8), (9), and (10) (L-168,049) in the chinese hamster ovary (CHO) cells expressing the hGlucR, IC_{50} values -/+ Mg^{2+} respectively, 490nM/n.d, 290nM/n.d, 80nM/800nM, 7nM/170nM; n.d., not determined.

further described. The binding affinity (IC_{50}) of this non-competitive inhibitor was described to be 3.7 ± 3.4nM (n=7) in the absence of divalent cations, but decreased 24-fold at physiological concentrations (5mM) of Mg^{++}. The compound did not inhibit binding of radiolabeled GLP-1 to the highly homologous human glucagon–like-peptide-1 receptor at concentrations up to 10μM. The binding domain of L-168,049 in the glucagon receptor was investigated by point mutations in the receptor. It was found that substitution of phenylalanine in position 184 of transmembrane domain 2, or tyrosine at position 239 in transmembrane domain 3, with alanine reduced the affinity of L-168,049 by 46- and 4.5-fold respectively, without affecting the affinity of labelled glucagon. These observations suggest that the binding sites for glucagon and L-168,049 are distinct, and thus correlates with the non-competitive functional data.

At the ACS (American Chemical Society) meeting in New Orleans in August 1999, Bayer presented biaryl and phenylpyridines as potent glucagon antagonists [118]. The initial hit (**11**) (Fig. **19**), found by screening, had affinity towards the hGluc receptor in the micromolar range. A huge SAR of this series of compounds has been investigated, but only published in the patent literature [119], and the series of biaryl and phenylpyridine derivatives have been optimised to compounds with high binding affinities in the low nanomolar range. Bayer has presented pharmacokinetic data as well as clinical data on their development candidate BAY 27-9955, but the exact structure of this development candidate has not yet been released [120-123]. BAY 27-9955 was reported to have nanomolar affinity towards the hGluc receptor (IC_{50} = 110nM), and to be a non-competitive antagonist (IC_{50} = 46nM). The affinity to glucagon receptors from other species is reduced compared to the affinity for the human receptor (Dog IC_{50} = 140nM; Mouse IC_{50} = 400nM, Rabbit IC_{50} = 700nM, Rat IC_{50} = 8000nM) which may explain why no animal pharmacodynamic data have been reported

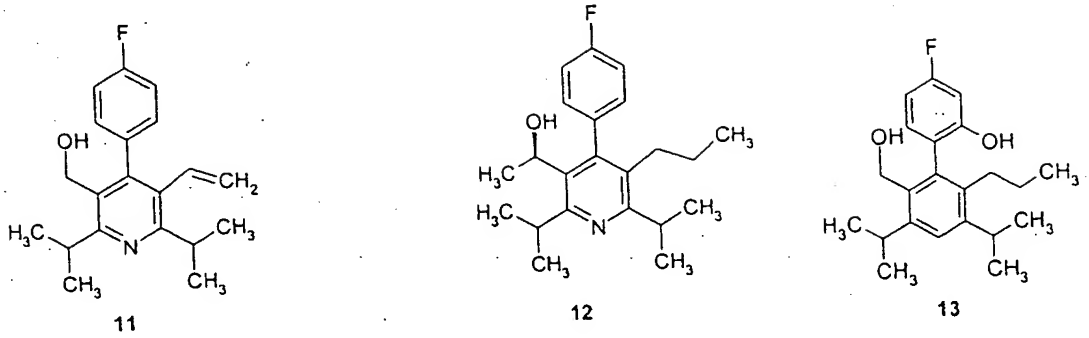


Fig. (19). Binding affinities of (11), (12), and (13) in the chinese hamster ovary (CHO) cells expressing the hGlucR, IC_{50} values respectively, 1100nM, 110nM, 110nM.

Clinical studies on BAY 27-9955 were presented at the EASD (European Association for the Study of Diabetes) meeting in Brussels, 1999 [123]. Sixteen young, healthy men were studied before and after a single dose (200 mg per oral) of a glucagon antagonist (BAY 27-9955). The subjects were fasted for 12h before the experiment, and infused with labelled glucose, in order to measure glucose production rates during the study. In order to suppress endogenous glucagon and insulin release, the subjects were infused with somatostatin during the study. Insulin was infused to replace basal insulin concentrations, and glucagon was infused in order to induce selective hyperglucagonaemia. The conclusion of the study was that during a physiological increase in plasma glucagon levels, BAY 27-9955 almost completely blocked the glucagon induced increase in HGP. However, Bayer has communicated that that the clinical studies of BAY 27-9955 have later been terminated.

At the ACS meeting in Washington in August 2000, Alanex and Novo Nordisk presented a new series of alkylidene hydrazides as potent glucagon antagonists [124, 125]. The original hit (**14**) (Fig. **20**), found in a high throughput screening, had micromolar affinity towards the hGluc receptor. Optimisation of this hit using a targeted library approach and traditional medicinal chemistry led to compounds with nanomolar affinities. One of the most potent compounds (**15**) (Fig. **20**) was found to be a non-competitive antagonist with high affinity (IC_{50} = 2.3nM), which was demonstrated to reduce glucagon-induced blood glucose increases in rats.

The Pfizer group has also recently described the effects of a fungal bisanthroquinone, skyrin [126], and shown that it can inhibit glucagon-stimulated cAMP production and glycogenolysis in rat hepatocytes and in primary cultures of human hepatocytes. Its effects were shared by oxyskyrin, but not by other bisanthroquinones. However, although the compound was shown not to interfere with signalling through other receptor types, it did not inhibit glucagon binding, but rather it interfered, in a selective manner, with the functional coupling of the receptor to adenylate cyclase [126].

Thus several different chemical series have been found which are antagonists at the human glucagon receptor and at least one compound (BAY 27-

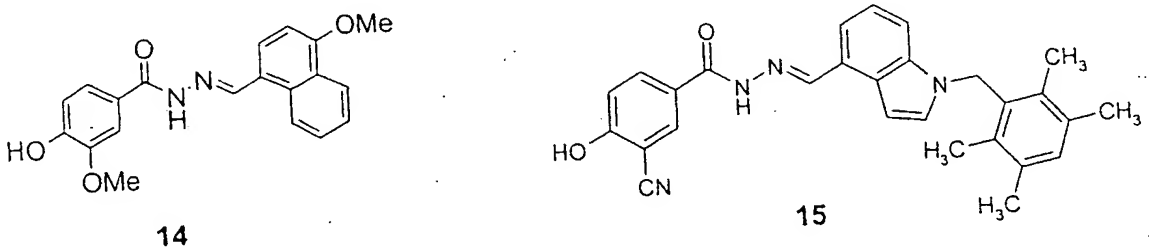


Fig. (20). Binding affinities of compounds (**14**) and (**15**) in the baby hamster kidney (BHK) cells transfected with hGlucR,

9955) has been published as having mechanistic proof-of-concept in man. Together with the other data described, and including the animal studies using the monoclonal anti-glucagon antibody, this suggests that this too may be an appropriate approach for novel anti-diabetic therapy.

CONCLUSIONS AND PERSPECTIVE

This article has shown that there are several viable targets being addressed to combat the elevated HGP observed in type 2 diabetes, and the animal efficacy data reviewed indicate that several development candidates should emerge. Indeed, and as already mentioned, a GP inhibitor (CP 368,296) has shown blood glucose lowering potential in Phase II clinical trials, and a glucagon antagonist (BAY-27-9955) has also shown mechanistic proof-of-concept in man; these would currently appear to be the furthest advanced of the approaches detailed herein. However, the next few years should provide further data in man as to the utility of such approaches.

Because the inhibition of HGP will also have many further metabolic consequences [126], it may be that the compounds targeting this would be adequate first line monotherapy for type 2 diabetes. In this regard, it is worth noting that in many of the animal studies described above full anti-hyperglycaemic efficacy has been observed, but, significantly, without any apparent induction of hypoglycaemia (e.g. [88-90, 106-109]), which would give such approaches a major advantage over existing therapies for type 2 diabetes. In addition, because there are no treatments currently specifically targeting HGP in the manners that we have described, such novel compounds, by their complementary mechanistic nature, should at the least provide valuable add-on therapy to existing treatments, especially if the key recommendations of the UKPDS in terms of trying to achieve as good blood glucose control as possible are to be reached [16]. The availability of selective pharmacological tools will also allow the determination of whether continuous reduction of HGP would be the best pharmacological approach, or whether for example overnight treatment or post-prandial treatment would be better [1-3, 5].

To summarise, therefore, the future appears to hold bright prospects for novel agents targeting HGP, which hence should provide type 2 diabetics (and perhaps also type 1 diabetics) with improved therapeutic options. There are other liver molecular targets other than those described which are known to influence hepatic glucose and fuel metabolism, e.g. GK which has already been mentioned [19], and there are also possibilities to alter HGP and other parameters by identifying key transcriptional factors which could revert to normal the aberrant liver enzyme expression patterns observed in type 2 diabetes [5, 6, 18, 19]. Another area which may receive more attention in the search for new approaches is the insulin signalling pathway, and insulin resistance mechanisms, in liver [127], as mice in which the insulin receptor has been specifically knocked out in this tissue develop a diabetes-like syndrome [128], in striking contrast to the corresponding specific knock out of the muscle insulin receptor [129]. This and many other studies demonstrate that the liver is clearly central to glucose handling and homeostasis, and that abnormal glucose handling by the liver is probably one of the key drivers of the diabetic state, and thus deserves the attention being directed towards it in the search for novel therapeutic approaches to diabetes.

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LIST OF ABBREVIATIONS USED (IN ORDER OF APPEARANCE)

HGP	=	Hepatic glucose production
UKPDS	=	United Kingdom Prospective Diabetes Study
PPAR	=	Peroxisome proliferator activated receptor
GK	=	Glucokinase
G-6-Pase	=	Glucose-6-phosphatase
G-6-P	=	Glucose-6-phosphate

ER	=	Endoplasmic reticulum
AICAR	=	5-Aminoimidazole-4-carboxamide riboside
ZMP	=	5′-Aminoimidazole-4-carboxamide ribonucleotide monophosphate
F-1,6-BPase	=	Fructose-1,6-bisphosphatase
GP	=	Glycogen phosphorylase
PP1	=	Protein phosphatase 1
DAB	=	1,4-Dideoxy-1,4-imino-D-arabinitol
NMR	=	Nuclear magnetic resonance
EGP	=	Endogenous glucose production
HbA1c	=	Glycosylated haemoglobin
GPCR	=	G-Protein coupled receptor
BHK	=	Baby hamster kidney
hGlucR	=	Human glucagon receptor
CHO	=	Chinese hamster ovary
GLP-1	=	Glucagon-like polypeptide 1
ACS	=	American Chemical Society
SAR	=	Structure activity relationship
EASD	=	European Association for the Study of Diabetes

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